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"On Protoplasmic Life." By F. Crace-Calvert, F.R.S.

A year since, the publication of Dr. Tyndall's interesting paper on the abundance of germ-life in the atmosphere, and the difficulty of destroying this life, as well as other papers published by eminent men of science, suggested the inquiry if the germs existing or produced in a liquid in a state of fermentation or of putrefaction could be conveyed to a liquid susceptible of entering into these states; and although at the present time the results of this inquiry are not sufficiently complete for publication, still I have observed some facts arising out of the subject of protoplasmic life which I wish now to lay before the Royal Society.

Although prepared, by the perusal of the papers of many workers in this field, to experience difficulties in prosecuting the study, I must confess I did not calculate on encountering so many as I met, and especially those arising from the rapid development of germ-life, of which I have hitherto seen no notice in any papers which have come under my observation. Thus, if the white of a new-laid egg be mixed with water (free from life), and exposed to the atmosphere for only fifteen minutes, in the months of August or September, it will show life in abundance. From this cause I was misled in many of my earlier experiments, not having been sufficiently careful to avoid even momentary exposure of the fluids to the atmosphere. To the want of the knowledge of this fact may be traced the erroneous conclusions arrived at by several gentlemen who had devoted their attention to the subject of spontaneous generation.

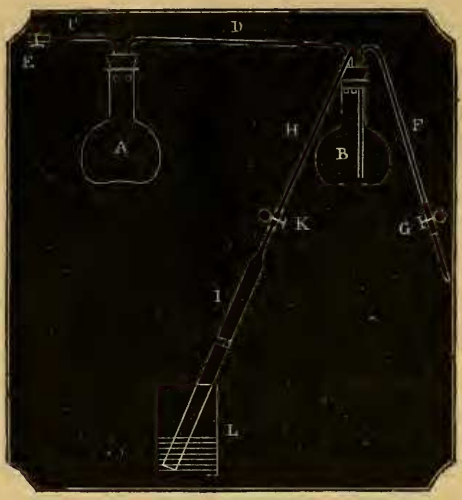
I believe that I have overcome the difficulty of the fluids under examination becoming polluted by impregnation by the protoplasmic life existing in the atmosphere, by adopting the following simple method of working.

As a pure fluid free from life, and having no chemical reaction, was essential to carrying out the investigation, I directed my attention to the preparation of pure distilled water. Having always found life in distilled water prepared by the ordinary methods by keeping it a few days, after many trials I employed the following apparatus, which gave very satisfactory results, as it enabled me to obtain water which remained free from life for several months.

It consists of two flasks, A and B (A rather larger than B), fitted with perforated caoutchouc stoppers*. These flasks are connected by the tube D. Into the stopper of A is fitted a tube C, to which is joined a piece of caoutchouc tubing, which may be closed by the

* The stoppers and caoutchouc tubing used for the various joints must be new, and must be well boiled in water before use.

clip E. Through the stopper of B is a siphon, F, the long limb of which is cut and joined with caoutchouc tubing, which can be closed by the clip G. Through this stopper is a third tube, H, connected



by caoutchouc with the tube I; this can be closed by the clip K. The tube I is about 3 feet long, and goes into the vessel L, which is partly filled with water.

The water to be distilled is mixed with solution of potash and permanganate of potash, and placed in the flask A*. Before distillation is commenced, a rapid current of pure hydrogen, or some other gas, must be passed through the apparatus by the tube C to displace the air and carry off all the germs the air may have contained. The clip G is first left open, then this closed and the clip K opened, which allows the gas to pass through the water in the vessel L.

The gas should be passed through for about fifteen minutes. The clip E is then closed, and the distillation carried on. When the operation is complete, the gas must be again passed through the apparatus, and the connexion with the tube I broken by closing the clip K. The water is drawn off through the siphon F. The long tube acts as a safety-tube, and is made so long that the absorption is noticed in ample time to close the clip before any air can enter through that tube.

The water has to be redistilled three or four times before it is obtained free from germs, and must be kept in the apparatus in which it is distilled until wanted, to prevent any contact with air.

Some water which had been distilled on the 20th of November,

* The reasons why I employed permanganate of potash (in large excess) were that under the influence of heat its oxidizing powers were much increased, and that it gave off no gas that could interfere with the purity of the water, this salt in solution not even yielding oxygen under any circumstances.

1870, being still free from life on the 7th of December, was introduced by the siphon H into twelve small tubes, and left exposed to the atmosphere for fifteen hours, when the tubes were closed. Every eight days some of the tubes were opened, and their contents examined. On the fifteenth, therefore, the first examination was made, when no life was observed; on the twenty-third two or three other tubes were examined, and again no life was detected; whilst in the series opened on the 2nd of January, 1871 (that is to say, twenty-four days from the time the tubes were closed), two or three black vibrios were found in each field.

Being impressed with the idea that this slow and limited development of protoplasmic life might be attributed to the small amount of life existing in the atmosphere at this period of the year*, a second series of experiments was commenced on the 4th of January. The distilled water in the flask being still free from life, a certain quantity of it was put into twelve small tubes, which were placed near putrid meat at a temperature of 21° to 26° C. for two hours, and then sealed. On the 10th of the same month the contents of some of the tubes were examined, when two or three small black vibrios were observed under each field. This result shows that the fluid having been placed near a source of protoplasmic life, germs had introduced themselves in two hours in sufficient quantity for life to become visible in six days instead of twenty-four. Other tubes of this series were opened on the 17th of January, when a slight increase of life was noticed; but no further development appeared to take place after this date, as some examined on the 10th of March did not contain more life than those of the 17th of January.

This very limited amount of life suggested the idea that it might be due to the employment of perfectly pure water, and that the vibrios did not increase from want of the elements necessary for sustaining their life. I therefore commenced a third series of experiments. Before proceeding to describe this series, I would call attention to the fact that the water in the flask had remained perfectly free from life up to this time, a period of close on sixteen weeks.

On the 9th of February 100 fluid grains of albumen from a new-laid egg were introduced, as quickly as possible and with the greatest care, into 10 ounces of pure distilled water contained in the flask in which it had been condensed and an atmosphere of hydrogen kept over it. On the 16th some of the fluid was taken out by means of the siphon H, and examined; and no life being present, twelve tubes were filled with the fluid, exposed to the air for eight hours, and closed. On the 21st the contents of some of the tubes were examined, when a few vibrios and microzymas were distinctly seen in each field. On the 27th other tubes were examined, and showed a marked increase in the amount of life. In this series life appeared

* During the intense cold of December and January last I found it took an exposure to the atmosphere of two days at a temperature of 12° C. before life appeared in solution of white of egg in the pure distilled water, whilst as the weather got warmer the time required became less.

in five days, and an increase in ten, instead of requiring twenty-four days, as was the case when pure water only was employed.

Albumen therefore facilitated the development of life. Of course the contents of the flask were examined at the same time; but in no instance was life detected. I believe that these three series of experiments tend to prove the fallacy of the theory of spontaneous generation; for if it were possible, why should not life have appeared in the pure distilled water, or in the albuminous solution, which were kept successively in the flask B, as well as in the fluids which were contained in the tubes, and had been exposed to the atmosphere or near animal matter in a state of decay, and had thus become impregnated with the germs of protoplasmic life? What gives still further interest to these experiments is, that, having operated during the severe weather of last winter, when little or no life existed in the atmosphere, I was able to impregnate the fluids with germs without introducing developed life.

The quantity of life produced in the above-recited experiments being comparatively small, I was led to infer that this might be due to the influence of the atmosphere of hydrogen employed to displace the air in the apparatus used for obtaining the water. I therefore, on the 2nd of March, prepared a solution of albumen similar to that before employed, but expelled the air out of the apparatus by pure oxygen; and as the contents of the flask B were free from life on the 8th of March, a series of small tubes were filled and exposed for twenty-six hours to the atmosphere near putrid matter, and then sealed. Several of these tubes were opened on the 11th, and immediately examined, when only a few cells were observed in each field. A second lot was opened on the 14th; and they showed considerable increase of life, there being two or three vibrios under each field. A third quantity was opened on the 25th, when no increase had taken place. This latter result tends to show that although oxygen appears to favour the development of germs, still it does not appear to favour their reproduction.

As the weather had become much warmer, and a marked increase of life in the atmosphere had taken place, some of the same albumen solution as had been employed in the above experiments was left exposed in similar tubes to its influence, when a large quantity of life was rapidly developed and continued to increase. This result appears to show that the increase of life is not due to reproduction merely, but to the introduction of fresh germs; for, excepting this fresh supply, there appears to be no reason why life should increase more rapidly in the open than in the closed tubes.

In concluding this paper I have great pleasure in recognizing the able and persevering attention with which my assistant, Mr. William Thompson, has carried out these experiments.